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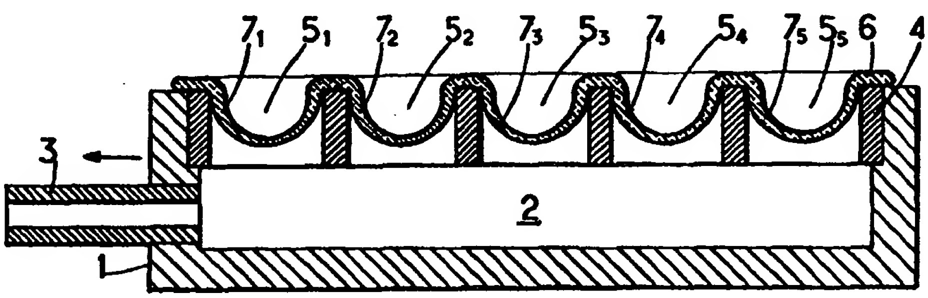
(71) Applicant *(for all designated States except US)*: CORNING INCORPORATED [US/US]; 1 Riverfront Plaza, Corning, NY 14831 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DANNOUX, Thierry [FR/FR]; 18, le Petit Bel Ebat, F-77210 Avon (FR). PUJOL, Gilbert [FR/FR]; 547, avenue Victor Hugo, F-77190 Dammarie-les-Lys (FR).

(74) Agent: HERZFELD, Alexander, R.; Corning Incorporated, Patent Dept., SP FR 02-12, Corning, NY 14831 (US).

(54) Title: METHOD AND DEVICE FOR THE MANUFACTURE OF A PLATE OF WELLS, NOTABLY FOR SAMPLES OF CHEMICAL OR BIOLOGICAL PRODUCTS



(57) Abstract

According to this method, a sheet made of a material in the plastic state is processed by thermoforming. A mold (4) is made, which is perforated with the pluralities of holes (5_i) distributed according to a distribution of wells to be formed in said sheet, said sheet is placed on said mold (4) and the sheet is heated to a temperature where it is in the plastic state, and c) between the two sides of the sheet, a gas pressure difference is established so that the material of said sheet, perpendicular to the holes of the mold, is aspired into these holes to assume the shape of wells (7_i) whose bottoms are separated from any contact with other surfaces.

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METHOD AND DEVICE FOR THE MANUFACTURE OF A PLATE OF WELLS, NOTABLY FOR SAMPLES OF CHEMICAL OR BIOLOGICAL PRODUCTS

BACKGROUND OF THE INVENTION

The present invention relates to a method and a device for the manufacture of a plate of wells, notably for samples of chemical or biological products and, more particularly, to such a plate made from a sheet made of a thermoformed material in the plastic state.

Such plates of wells are known to carry out biological tests or cultures, and they are made from a sheet of organic plastic material (for example, polycarbonate) which has been thermoformed. The samples contained in the wells often have to be observed with a microscope through the bottom of the wells, notably when these samples contain living cells which collect at the bottom of these wells as a result of gravity. Plates made of organic plastic material are generally not suitable for such observation because of unfavorable optical properties such as birefringence, fluorescence of the material, surfaces without optical finish, etc.

To overcome this drawback, there is a so-called "microtitration" plate which is made of injection-molded plastic material, this plate comprising a two-dimensional distribution of bottomless recesses. A plate of polycarbonate of optical quality is glued against all the recesses so as to form the bottoms of what become individual wells. Clearly, such a plate allows

observations with a microscope through the bottoms of the wells, but it presents the drawback of requiring the gluing of the polycarbonate plate by an adhesive product which may be toxic to cell cultures. Gluing plates together also negatively impacts the cost of the manufacture of the plate.

SUMMARY OF THE INVENTION

The purpose of the present invention is to make a plate of wells which presents none of these drawbacks and which, consequently, permits observations with a microscope, while at the same time being inexpensive to manufacture.

This purpose of the invention is achieved, as well as others which will become apparent upon reading the following description, by a method for the manufacture of a plate of wells of the type described in the preamble of the present description. The method comprises the steps of:

a) providing a mold, which is perforated by a plurality of holes arranged according to a distribution of wells to be formed;

b) placing a sheet of thermoformable material on said mold and heating said sheet to a temperature where it is in the plastic state, and

c) establishing a gas pressure between the two sides of the sheet, so that the material of said sheet, being perpendicular to the holes of the mold, is aspired into these holes thereby assuming the shape of wells whereby the bottoms of the wells are separated from any contact with other surfaces of the mold.

As will be seen below, this method allows the two opposite surfaces of the bottom of a well to preserve an optical quality surface or finish state which is compatible with observation through this bottom, this optical finish remaining intact because during the molding, any contact between the bottom of the well and

other surfaces capable of altering its surface state is avoided.

For the implementation of this method, the invention provides a device comprising a) a mold having a side capable of receiving a sheet of the thermoformable material, said mold being perforated, perpendicularly to this side, by a plurality of holes, b) an enclosure delimiting with said mold a space, c) means to heat the sheet and to bring it to the plastic state, and d) a low-pressure gas generator which can selectively be connected to said space to thermoform wells in said sheet, by aspiration into said holes of the material of said sheet located perpendicular to said holes.

The method according to the invention allows the making of a plate of wells comprising a distribution of thermoformed wells in a sheet made of a thermoplastic material, which plate is characterized in that two sides of the wall of the wells formed in the sheet present an optical finish. The thermoplastic material used is preferably an inorganic glass.

Other characteristics and advantages of the present invention will become apparent upon reading the following description.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagram of a first embodiment of a device for the implementation of the method according to the invention.

Figure 2 is a perspective view of a part of a plate of wells obtained using the device of Figure 1.

Figure 3 is a cross-sectional view of the plate according to the invention, mounted on a frame adapted to this plate.

Figure 4 is a diagram of a second embodiment of a device to implement the method according to the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference is made to Figure 1 of the drawing in the appendix where a cross-sectional representation is given of an enclosure 1 delimiting a space 2 which can be placed in communication with a low-pressure generator (not shown) through the intermediary of a pipe 3. The enclosure is closed by a mold 4 which assumes the general shape of a planar plate perforated by cylindrical holes 5_1, 5_2,..., 5_i, etc., having axes perpendicular to its surface. These holes can also be conical and in that case they have a conicity of 5-20°. They can have a circular or polygonal section, for example, square, rounded at the edges. The holes preferably have identical geometry and they are arranged in a dense regular two-dimensional distribution, in lines and in columns.

To make a plate of wells according to the invention, the enclosure 1 is placed in an oven, after having placed a sheet of thermoplastic material such as an inorganic glass or an organic material such as a polycarbonate on the mold 4. The surface state of the sheet must be of compatible optical quality with that which is desired for the walls of the bottoms of the wells to be made. The oven is heated at a temperature where the glass reaches a viscosity compatible with a thermoforming operation. Then a low pressure is established in the space 2, compared to the gas pressure existing in the oven, and notably at the level of the surface of the plate opposite that which faces the space 2. Due to the gas pressure difference, the glass plate is deformed in the areas of this plate covering the holes 5_i. These areas are aspired towards the space 2 and they then gradually assume an approximately hemispherical shape.

According to an important characteristic of the present invention, the temperature of the plate, the duration of application of the low pressure in the enclosure 1 and the value of this low pressure are adjusted so that during the course of their progressive

deformation, the aspired areas of the plate remain constantly separated from the surfaces of the hole and from any other surface in general. In this manner, the manufacture of a plate 6 of wells 7_1, 7_2,..., 7_i, etc., is ensured, as represented in cross section in Figure 1, the two sides of the wall of each well presenting a perfect optical finish because of the absence of any contact between these surfaces and the surfaces of the mold during the operation of forming these wells by thermoforming under a vacuum.

It can be understood that the optical finish so obtained is very advantageous in the observation of biological cultures, for example, through the bottoms of the wells where these cultures are carried out. This is particularly the case when the material used to make the plate is a material of optical quality, such as an organic or inorganic glass. For biological cultures, the selection of a mineral glass is particularly appropriate, because of its chemical inertness.

It should also be noted that the plate of well 6 according to the invention presents wells whose wall is thinner at the bottom of the wells than at their opening because of the vacuum thermoforming method used to make these wells. The method of manufacture according to the invention thus allows the making of plates with wells having a small bottom thickness, less than 0.2 mm, for example, compatible with observations using a microscope with a very short focal length, such as, for example, confocal microscopes. In Figure 3, the lens 20 of such a microscope is shown in the position of observation of a bottom of a well, through a drop 21 of an index-adapting liquid.

As an illustrative and nonlimiting example, an embodiment of the plate of wells according to the invention will now be described, as it appears in Figures 2 and 3. The plate is made from a planar glass sheet referenced 0211 in the catalogues of the company Corning Incorporated, this plate covering a surface of 80 mm x 116

mm and comprising 96, 384, or 1536 wells arranged in 8, 16, or 32 adjacent rows of 12, 24, or 48 wells, respectively. The plate can also have the standard dimensions of a microscope slide (26 x 76 mm) and then comprise, for example, 60 or 250 wells. The separation of the wells is compatible with the usual means for multichannel distribution with pipettes.

To thermoform such plates, a mold 4 is made of a refractory alloy referenced NS 30 in the catalogues of the company UGINE (AFNOR Z12CN25-20), this mold being perforated by holes having a depth of 2 mm and a diameter of 2 mm, these holes being separated by walls having a thickness of at least 0.25 mm and advantageously presenting a clearance angle of 5-20°C [sic]. Such holes can be obtained by mechanical or chemical machining. Over the surface of the mold which receives the glass sheet, an alcohol suspension of powdered calcium hydroxide is prepared, the latter having a granular size of less than 1 mm, and a dispersant is added, such as the agent DISPERBYK 190 from the company BYK Chemie (Germany). This suspension facilitates the separation of the plate of wells and the mold, after the thermoforming of this plate. Being applied against the top surface of the mold, this suspension does not affect the surface state of the bottom of the wells.

The planar glass sheet is mounted on the mold 4 and the plate/mold/enclosure assembly is placed in an oven, the latter is heated to a temperature of approximately 675-695°C, and this temperature is maintained for approximately 5-7 min, while applying a 0.75-bar low pressure to the space 2.

The starting planar glass sheet preferably has a thickness of 0.3 mm. The bottoms of the wells present, as seen above, a smaller thickness, in this instance approximately 0.160 mm. Such a thickness is compatible with the focal length of the lenses of microscopes, notably confocal microscopes, which routinely are 0.2 mm.

Since the wells are formed and the temperature of the plate is such that the rigidity of the plate is sufficient

(or approximately 550-600°C), the plate is separated from the mold by establishing a slight excess pressure in the space 2, before the cooling of the mold can hold and damage the plate.

Figure 2 represents a fraction of the surface of the plate obtained, as one can visualize it using a microscope with mechanical scanning. The generally hemispherical shape of the well 7_i, 7_{i+1}, etc., appears in this figure, which also shows that the openings of the wells are connected to each other by surfaces with a progressive curvature, without discontinuities. This geometry is advantageous from at least two points of view. It prevents air bubbles from being trapped on the surface when cultures in a liquid medium are distributed in the wells. It also promotes the collection of live cells at the bottom of the wells as a result of gravity, as will be seen below.

Two methods can be used to distribute cultures in a liquid medium or, more generally, any liquid, in the different wells. The first consists in using standard micropipettes, separated or in sets. The second method consists in depositing on the plate a drop which simultaneously covers several adjacent wells. By gravity, this liquid spreads over the plate possibly filling other adjacent wells. If the liquids contain plant or animal cells, one can note that they distribute themselves as a result of gravity between the wells and they advantageously regroup at their bottoms.

It is possible to apply to the surface of the plate, around the openings of the wells, a hydrophobic product such as a silane, for example. Such a treatment of this surface contributes to the filling of the wells by preventing any liquid from remaining between them. It also avoids any communication of liquid between two adjacent wells after the filling of these wells. This treatment permits the handling, without particular precaution, of a plate of 250 or 1536 wells having a diameter of 2 mm, for

example, and such a plate can even be turned upside down for hanging-drop cultures, for example.

The plate 6 of wells according to the invention may be supported by means of an emptied frame 8 presenting an edge 8' against which the periphery of the plate 6 is rested, as shown in Figure 3. The plate 6 can possibly be glued or welded to the frame 8, at the level of the edge 8'. The frame 8 has a height which is greater than the thickness of the plate 6 so as to protect the bottom of the wells from any contact with a surface on which the frame is placed. The latter also facilitates the manual or automatic handling operations of the plate according to the invention.

Figure 4 is a representation of another embodiment of the device for the implementation of the method according to the invention, which is adapted to continuous production, and is also advantageous from an economical point of view. The device comprises a reservoir (not shown) of a molten glass which discharges a sheet of glass 19 having an adjusted viscosity through a slit 9. This sheet is taken up by a cylindrical drum 10 which is mounted so it can rotate around the fixed shaft 11 and so it is driven by means (not shown) at a predetermined speed.

The drum 10 is integrally connected to a cylindrical core 12 which is internal to the drum. The surface of this core is slightly separated from the internal surface of the drum 10, resting against axial ribs such as 13_1, 13_2, etc., formed, for example, on the core 12.

For its part, the fixed shaft 11 is hollowed out in the form of peripheral chamber 14 which communicates, through the intermediary of a radial channel 15 and an axial channel 16 with a low-pressure generator (not shown). When the drum 10 turns, the chamber 14 communicates at each instant with one of several radial channels, such as 17_1, 17_2, 17_3, etc., by one of the annular chambers such as 18_1, 18_2, etc., present between the drum 10 and the core 12, at the level of the ribs 13_i.

The drum 10 plays the role of a mold for the sheet of glass 19 which envelopes this drum. For this purpose, the drum is perforated by holes $5'_i$, just like the mold 4 of Figure 1. The low-pressure generator being connected to the pipe 16, the sheet 19 of glass which continually descends from the slit 9 is grabbed by aspiration by the surface of the drum, this sheet then enveloping this drum over approximately one-fourth of its surface. The sheet 19 is subjected to a low pressure at the level of its side which is in contact with this drum, thanks to the pipe 17_i which transmits, at a given instant, a low pressure to the corresponding rotatory annular chamber 18_1, 18_2. It can be seen that the holes $5'_i$ perforated into the drum 10 then play the role of the holes 5_i of the mold of Figure 1, in the formation of wells in the sheet of glass 19, by separating the surface of these wells from any contact during their formation, as seen above in connection with Figure 1. The flow rates of glass exiting the hopper 9, the speed of rotation of the drum, the temperature and the thickness of the sheet 19 as well as the low pressure applied to this sheet are adjusted so that essentially hemispherical wells are obtained, similar to those represented in Figure 1. The sheet so deformed leaves the drum 10 to be then cut at a high temperature and then cooled by means which are not shown, to form section 6_i, 6_{i+1}, etc., which are each capable of constituting a plate of wells according to the invention.

It is clear that the device of Figure 4 ensures a continuous production of plates, which production is better adapted than the device of Figure 1 to large production volumes. For such a production, the temperature of the glass can be in the range of 740-760°C, and the contact time between the glass sheet and the drum on the order of only 5-20 sec. The temperature of the drum is maintained, by means which are not shown, at approximately 550-600°C.

It now appears that the invention indeed allows achieving the set purpose, that is to ensure the

manufacture at reduced cost of plates of wells which allow the observation with a microscope of the content of the wells through their bottoms. Besides the previously mentioned advantages of the plate according to the invention, the plate also presents the advantage of potentially containing a variable number of wells, which can be very high, the manufacturing method described being suitable for small-volume wells, for example, 0.2-10 mL. With a very large number of wells, it is possible to simultaneously treat a great number of samples, for example, 100,000 samples/day, as is done routinely today. In addition, the wells of the plate according to the invention, because of their geometry and their discontinuity-free connections lend themselves to the formation of a convex meniscus of the contained liquid in each well. When the liquid contains live cells, they thus benefit from a maximum surface for exchanging oxygen with the air, which is favorable to their development. In addition, the contamination of the samples contained in the wells by toxins borne by the internal surface of these wells, and also the dissipation of any solvents contained in the wells through the walls, are particularly important if the well has a small volume. The use according to the invention of glass with high chemical inertness to make plates of such wells with small volume is then particularly advantageous to minimize this problem.

As seen above, the present invention allows the making of plates of wells with essentially hemispherical bottoms. In the case of syntheses of molecules on beads, placed in such wells, this form advantageously allows a reduction of the volumes of reagent coating the beads.

Naturally, the invention is not limited to the described and represented embodiments which are given only as examples. Thus, the plate according to the invention can be used to carry out tests on samples of chemical products and not only biological products or materials. In addition, although inorganic glass is preferred to make this plate because of its above-mentioned chemical

inertia, other mineral or organic thermoplastic materials, for example, could be used. In addition to transparent materials of optical quality, other materials can be used if no observation through the bottom of the well is to be carried out.

What is claimed is:

1. A method for the manufacture of a plate of wells, designed to contain samples of chemical or biological products, by thermoforming of a sheet of a material in the plastic state, comprising the steps of:

a) providing a mold (4;10) having a plurality of holes distributed according to a distribution of wells to be formed in said sheet,

b) placing a sheet of thermoformable material on said mold (4;10), and heating the sheet to a temperature where it is in a plastic state, and

c) establishing a gas pressure between the two sides of the sheet so that the material of said sheet, being perpendicular to the holes of the mold, is aspired into these holes thereby assuming the shape of wells, $(5_i;5'_i)$ whereby the bottoms of the walls are separated from any contact with other surfaces of the mold.

2. A device for the implementation of the method according to Claim 1, characterized in that it comprises a) a mold (4;10) having a side capable of receiving a sheet of thermoformable material, said mold being perforated, perpendicular to that side, with a plurality of holes $(5_i;5'_i)$, b) an enclosure (1;12) delimiting with said mold (4;10) a space $(2;18_i)$, c) means to heat the sheet and to bring it to the plastic state, and d) a low-pressure gas generator which can be selectively connected to said space (2;18) to thermoform well (7_i) in said sheet, by aspiration into said holes of the material of said sheet located perpendicular to said holes.

3. Device according to Claim 2, characterized in that the shape of the holes $(5_i,5'_i)$ perforated into the mold (4;10) is selected from: a cylindrical, [or] conical shape, with circular or polygonal cross section.

4. Device according to any one of Claims 2 and 3, characterized in that the holes (5_i;$5'_i$) are distributed two dimensionally in a regular manner.

5. Device according to any one of Claims 2-4, characterized in that the surface of the mold (4) which receives said sheet is generally planar and it receives an individualized sheet.

6. Device according to any one of Claims 2-5, characterized in that it comprises means to establish a low pressure in the space (2) which is capable of separating a molded plate from its mold (4).

7. Device according to any one of Claims 2-4, characterized in that the surface of the mold (10) which receives said sheet is cylindrical, in that this surface turns continuously around its axis to receive a sheet of material in the plastic state (19) which is continually discharged by a source (9) over a part of its surface, the sheet leaving this surface to deliver a continuous sheet containing wells.

8. Device according to Claim 7, characterized in that it comprises means to continually cut the delivered sheet by the surface of the mold (10) so as to form plates of wells.

9. Plate of wells obtained by the implementation of the method according to Claim 1, comprising a distribution of thermoformed wells in a sheet made of a thermoplastic material, characterized in that the two sides of the wall of the well (7_i) formed in the sheet present a surface state of optical quality.

10. Plate according to Claim 9, characterized in that it consists of inorganic glass.

11. Plate according to any one of Claims 9 and 10, characterized in that its thickness increases from the bottom to the opening of the wells.

12. Plate according to Claim 11, characterized in that its thickness at the bottom of the wells is less than 0.2 mm.

13. Plate according to any one of Claims 9-12, characterized in that it bears, around the opening of the wells, a hydrophobic coating.

14. Plate according to any one of Claims 9-13, characterized in that it comprises a regular two-dimensional distribution of wells.

FIG.: 1

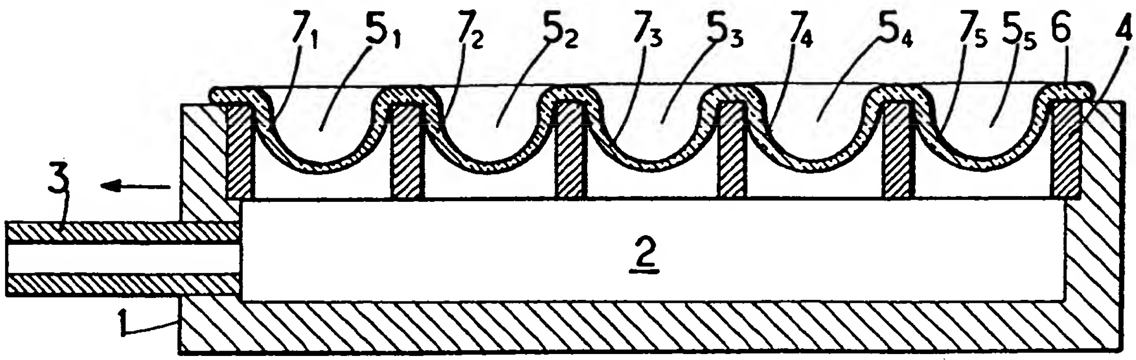

FIG.: 2

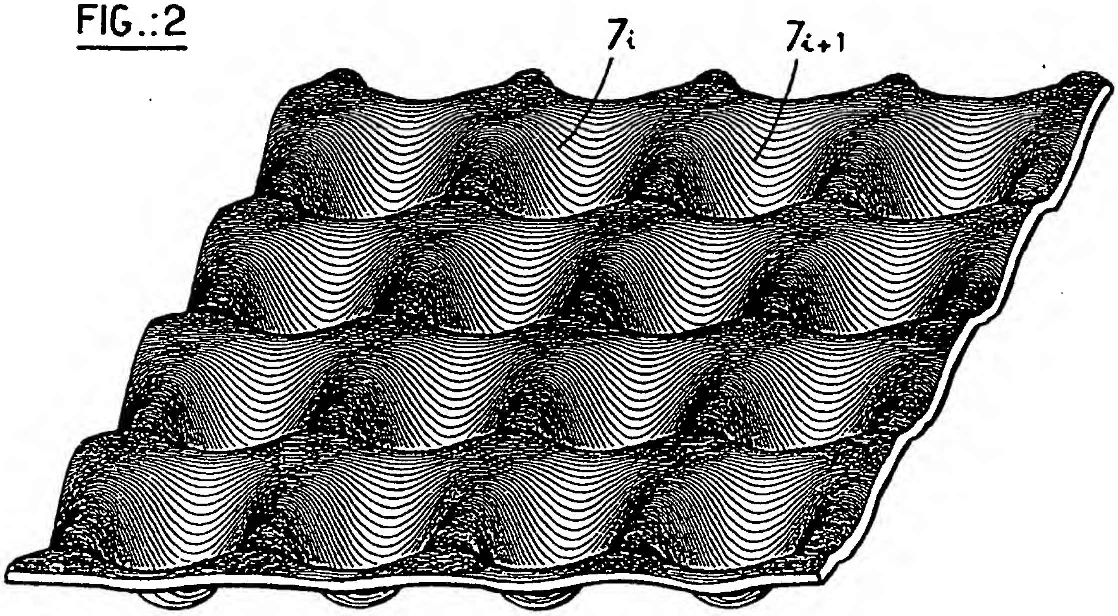

FIG.: 3

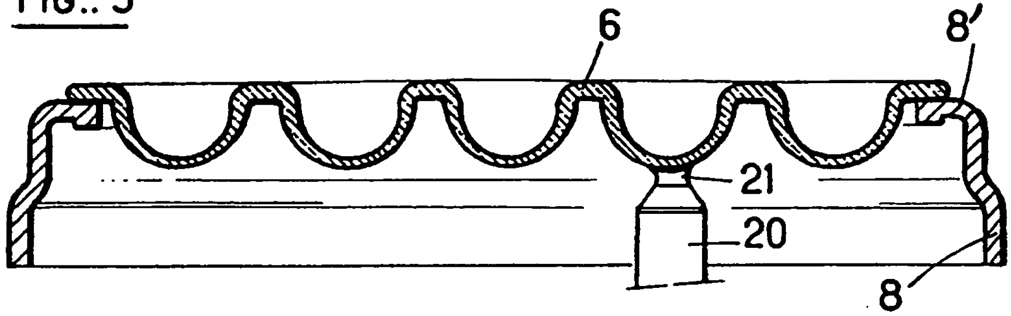
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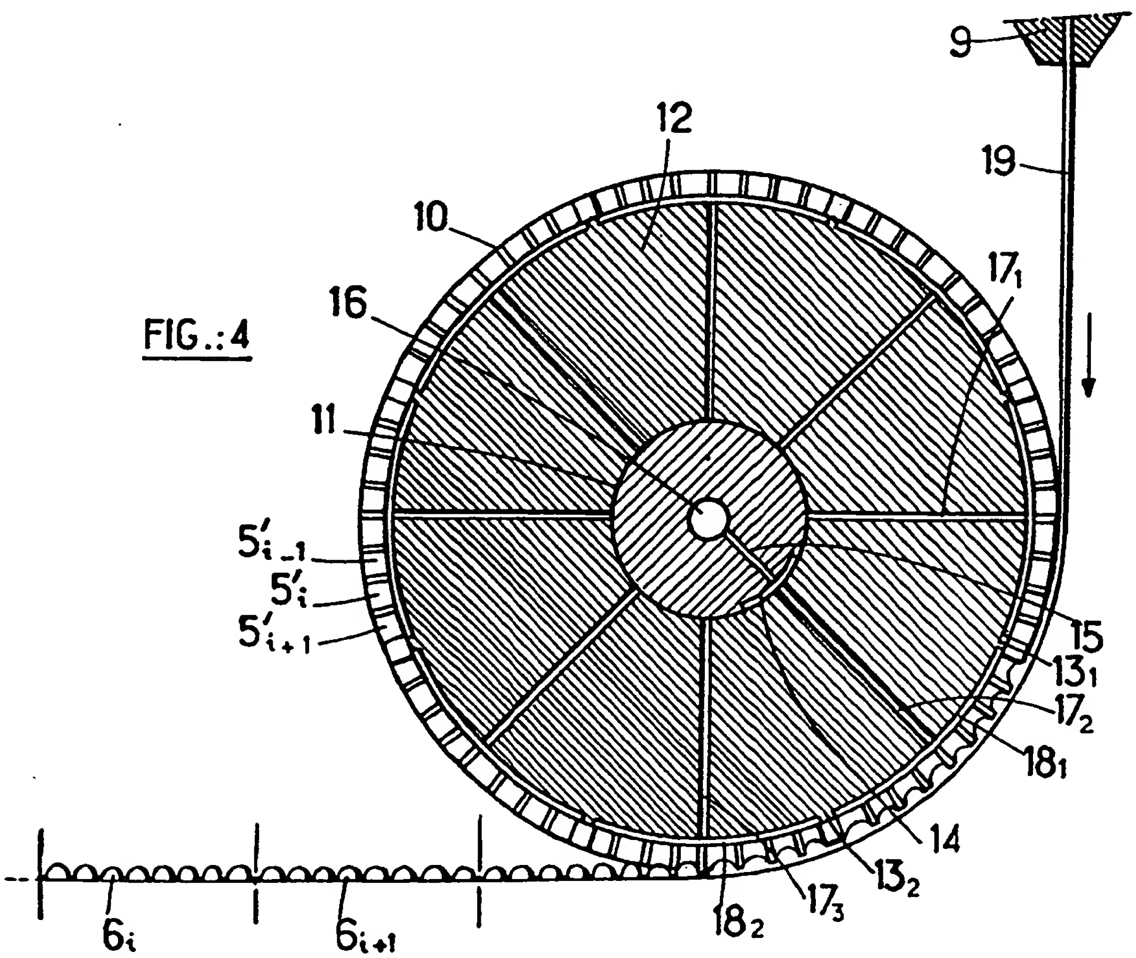
FIG.: 4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17₃
18₂
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/19280

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L3/00 B29C51/22

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L B29C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	DE 17 04 211 A (MASCHINENFABRIK GENG & CO) 22 April 1971 see the whole document	7

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

I. national Application No
PCT/US 97/19280

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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Information on patent family members

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